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MATURATION OF PENAEID SRHIMP: LIPIDS IN THE MARINE FOOD WEB

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ABSTRACT

We have determined that certain lipids are required in the diets of penaeid shrimp to promote ovarian maturation. In an attempt to identify appropriate feedstock supplements which contain these lipids, we have examined several invertebrate species from two locations: West Bay, Galveston, and the Gulf of Mexico 50 km offshore from Galveston. At each location, with a few notable exceptions, the lipid profiles for the various species are very similar. This finding implies that many lipids pass through the food web unaltered and that the suitability of a particular invertebrate for inducing ovarian maturation may depend upon the diet of that invertebrate.

INTRODUCTION

Our approach to obtaining reproducible ovarian maturation and spawning with animals held in captivity has been based largely upon manipulation of the lipid content of the diet (Middleditch et al. 1979d). Our hypothesis was that essential fatty acids not present in previously employed feedstocks were required to promote maturation, since such substances were present in relatively large quantities in mature female shrimp which were about to spawn in the wild (Middleditch et al. 1980). Indeed, when the diets of *Penaeus setiferus* (Brown et al. 1979; Lawrence et al. 1980) and *P. stylirostris* (Brown et al. 1980) were supplemented with marine invertebrates containing relatively high concentrations of essential fatty acids, ovarian maturation and spawning followed. These experiments have only indicated, rather than proved, that essential fatty acids are implicated in the maturation process. One approach to resolving this problem has been to examine the lipid content of components of the marine food web which are available to adult shrimp. This work is also useful in identifying other appropriate feedstock supple-

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ments for the animals which are maintained in captivity.

Two sets of data are presented here. The first is for the fatty acid and sterol content of a variety of invertebrates collected from a coastal bay. The second is for the sterol content of a different group of invertebrates collected from the vicinity of an offshore oil platform.

MATERIALS AND METHODS

Plankton, barnacles (*Balanus tintinnabulum*), white squid (*Loliguncula brevis*), sea urchins (*Arbacia unctulata*), brittle worms (*Choloeia viridis*), brown shrimp (*P. aztecus*), pink shrimp (*P. duorarum*), sugar shrimp (*Trachypenaeus similis*), and mantis shrimp (*Squilla empusa*) were collected from the region of the Buccaneer Oil Field in the northwestern Gulf of Mexico, 50 km south of Galveston, Texas, in the summer of 1976. No attempt was made to separate and analyze individual species of plankton; a copepod (*Acartia tonsa*), the sergestid shrimp (*Lucifer faxoni*), and a chaetognath (*Sagitta tenuis*) were among the major species obtained (Fotheringham and Brunenmeister 1975). Plankton were obtained using paired 61 cm diameter bongo nets with mesh sizes of 0.333 and 0.505 mm. Timed double oblique tows were performed to sample the depth strata equally from the surface to about one meter from the bottom. Barnacles, sea urchins, and worms were collected by hand by divers. Shrimp and squid were obtained by trawling. Samples were frozen for transport to the laboratory, and were kept frozen prior to analysis.

A second group of marine invertebrates comprising five annelids (a blood worm, *Glycera* sp.; a red-legged worm, *Marphysa sanguina*; a rag worm, *Nereis succinea*; a bearded worm, *Pista palmata*; and a tube worm, *Spirochaetopterus oculatus*), four bivalves (a mussel, *Brachiodontes recurvus*; an oyster, *Crassostrea virginica*; an angelwing clam, *Cartopleura costata*; and a razor clam, *Tagelus plebeius*), two crustaceans (a barnacle, *Balanus* sp.; *Uca minax*), and a gastropod (a sharkeye snail, *Nassarius vibex*) was collected by hand from West Bay, Galveston, Texas, in the spring of 1978. Again, samples were kept frozen prior to analysis.

Similar analytical procedures were used for the examination of all the samples. Tissue samples were homogenized and saponified. Sterols were extracted using diethyl ether. For those samples where fatty acids were also examined, the aqueous phase was acidified and a similar extraction procedure yielded a fraction containing the acids. Fatty acids were converted to methyl esters prior to gas chromatography (GC) or combined gas chromatography-mass spectrometry (GC-MS). Sterols were examined as trimethylsilyl (TMS) ethers.

RESULTS

The major fatty acids for the specimens from West Bay, together with their relative concentrations, are given in Table 1. Data for the sterols in these samples are given in Table 2. Sterol data for the samples from the Buccaneer Oil Field are given in Table 3. The hydrocarbon data for the Buccaneer Oil Field samples have been reported elsewhere (Middleditch et al. 1977, 1979a,b,c).

Table 1. Major Fatty Acids in Marine Invertebrates Collected from West Bay, Galveston, Texas, during Spring 1978[a]

Specimen	16:0	16:1	18:0	18:1	20:1	20:4+5[b]	22:5+6[c]
Annelids:							
Glycera sp.	11.2	3.1	8.7	6.4	12.2	14.0	12.9
Marphysa sanguina	11.2	3.6	7.6	7.0	9.1	21.1	18.0
Nereis succinea	16.9	20.9	4.3	9.4	4.2	14.8	2.7
Pista palmata	18.5	26.5	2.7	8.2	3.6	18.3	5.0
Spirochaetopterus oculatus	19.0	8.6	7.1	6.5	7.7	19.8	5.1
Bivalves:							
Brachiodontes recurvus	23.7	11.5	4.1	8.0	8.6	10.0	10.7
Crassostrea virginica	23.6	5.5	3.7	8.8	6.8	18.1	12.7
Cartopleura costata	22.2	16.8	6.8	6.2	4.0	20.6	9.2
Tagelus plebeius	25.6	15.9	9.1	10.3	10.7	3.0	3.7
Crustaceans:							
Balanus sp.	21.2	23.3	4.3	11.0	7.8	7.3	4.2
Uca minax	14.9	13.4	6.7	17.9	10.5	16.5	6.4
Gastropod:							
Nassarius vibex	13.9	7.6	9.2	9.7	12.0	15.7	8.5

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]20:4 and 20:5 not resolved.

[c]22:5 and 22:6 not resolved.

Table 2. Major Sterols in Marine Invertebrates Collected from West Bay, Galveston, Texas, during Spring 1978

Specimen	A	B	C	D	E	F	G	H	I	J
Annelids:										
Glycera sp.		3.6	89.0		3.1	4.4	1.3	0.9[a]		
Marphysa sanguina		3.1	68.7		7.6	7.6	1.7	11.3		
Nereis succinea		3.1	76.3		9.2	3.8	2.7	3.1		1.9
Pista palmata	1.3	5.0	62.7		10.0	8.8	2.5	7.5		
Spirochaetopterus oculatus	1.1	8.3	75.5		6.4	4.5	1.5	0.8		
Bivalves:										
Brachiodontes recurvus	3.7	8.3	46.2		23.1	9.5	4.6	4.6		
Crassostrea virginica	5.9	8.2	29.7		21.5	17.2	5.3	10.5	1.5	
Cartopleura costata	5.9	5.6	29.6		19.2	18.0	6.5	12.1	1.8	1.2
Tagelus plebeius	5.8	12.0	26.2		22.0	22.5	2.6	8.9		
Crustacean:										
Balanus sp.	1.5	5.1	50.5	29.8	2.5		1.5	1.0	5.6	
Gastropod:										
Nassarius vibex	2.8	5.0	55.6		17.8	8.1	4.7	6.1		

A = 22-*trans*-24-norcholesta-5,22-dien-3β-ol; B = 22-dehydrocholesterol; C = cholesterol; D = desmosterol, E = 24-methylcholesta-5,22-dien-3β-ol; F = 24-methylcholesterol; G = stigmasterol; H = sitosterol; I = 24-propylidenecholesterol; J = C_{29}-sterol.

[a]Also contains 24-ethylidenecholesterol.

Table 3. Major Sterols in Marine Invertebrates Collected from the Region of the Buccaneer Oil Field during Summer 1976

Specimen	A	B	C	D	E	F	G	H	I	J
Plankton:										
Mixed zooplankton	3.8[a]	13.8	62.4	14.3[b]	3.8[c]			1.9[d]		
Squid:										
Loliguncula brevis		2.0	98.0							
Sea urchin:										
Arbacia unctulata	2.4	6.5	80.6	10.5[b]						
Polychaete worm:										
Choloeia viridis	1.7	7.7	85.5	3.4[b]	1.7[e]					
Barnacle:										
Balanus tintinnabulum	1.2	11.6	60.8	24.9	1.5					
Shrimp:										
Penaeus aztecus		2.0	98.0							
Penaeus duorarum	1.0	1.9	95.2	1.9						
Trachypenaeus similis		2.0	98.0							
Squilla empusa	1.0	2.9	96.1							

A = 22-*trans*-24-norcholesta-5,22-dien-3β-ol; B = 22-dehydrocholesterol; C = cholesterol; D = desmosterol; E = 24-methylcholesta-5,22-dien-3β-ol; F = 24-methylcholesterol; G = stigmasterol; H = sitosterol; I = 24-propylidenecholesterol; J = C_{29}-sterol.

[a]Also contains 22-*trans*-24-norcholesta-22-en-3β-ol.

[b]Also contains 24-methylcholesterol.

[c]Also contains 24-methylcholestadienol.

[d]Also contains 24-ethylidenecholesterol.

[e]Also contains 22-dihydroergosterol.

Identification of the fatty acids (as their methyl esters) was based upon their gas chromatographic retention times and their mass spectra. The data presented in Table 1 were obtained using OV-1 or OV-101 as stationary phases. Under these conditions, most of the major fatty acid methyl esters are separated and individually determined. However, certain pairs of essential fatty acid methyl esters are not. These include 20:4/20:5 and 22:5/22:6. Moreover, such pairs of compounds also give very similar mass spectra. Thus, we have reported only the total concentrations of these compounds. Subsequent examination of selected samples on more polar stationary phases (SP-2340 or Silar-10C), which do separate the closely similar essential fatty acids, has confirmed that each of the suspected compounds was indeed present (Missler 1979).

Sterols of invertebrates from the region of the Buccaneer Oil Field were identified from data obtained for both the free sterols and their TMS ethers using packed GC columns. Data for the invertebrates from West Bay were obtained only for sterol TMS ethers, but employing capillary columns. In each case, GC-MS was used for the final identification (Brooks and Middleditch 1973).

DISCUSSION

INVERTEBRATE FATTY ACIDS FROM WEST BAY

Examination of the data in Table 1 indicates that, in most samples, the unsaturated fatty acids are the major fatty acids. The C_{20} compounds are particularly abundant. Relatively low concentrations of the C_{18} compounds are observed. Most of the specimens were collected from Confederate Reef, an oyster-shell reef which is exposed at low tides. However, the bivalves were collected from a mud bank or from shallows with a muddy bottom about 500 m to the southwest. It is of interest to note that, in these specimens, palmitic acid (16:0) predominated. Barnacles were collected from wooden posts in the same region of West Bay as the bivalves; the major fatty acid in these specimens was palmitoleic acid (16:1). There is little published information on the fatty acid composition of invertebrates from coastal regions of the Gulf of Mexico. However, there is considerable evidence to support the contention that fatty acids migrate through food webs with only minor modification of their relative abundances (for a discussion, see Middleditch et al. 1980). In the present study, we find one distribution of fatty acids to be typical of invertebrates collected on an oyster-shell reef and a second, quite different, distribution from a muddy habitat only 500 m away. Further work is required to determine whether the bivalves and barnacles examined have inherently different fatty acid profiles, but we have shown that the annelids, the other crustacean, and the gastropod all contain fatty acids in similar relative concentrations.

INVERTEBRATE STEROLS FROM WEST BAY

The trends in sterol content of these specimens are even more striking. The samples collected from Confederate Reef all had very similar steroid profiles. Cholesterol predominated, and the C_{28} sterols were usually the next most abundant. In contrast, the bivalves from the muddy habitat contained much larger amounts of C_{28} sterols, while cholesterol accounted for only 26 to 46% of the total sterols. The C_{28} compounds are typical plant sterols, and their presence in the specimens feeding at the muddy location would presumably reflect the abundance of vegetation in this habitat. In contrast, Confederate Reef is devoid of vegetation, and the relative concentrations of C_{28} sterols in specimens from that location are correspondingly low. The barnacles have a quite different steroid profile, with cholesterol accounting for about 50% of the sterols and desmosterol for most of the remainder. Barnacles typically contain relatively high concentrations of desmosterol (Fagerlund and Idler 1957).

INVERTEBRATE STEROLS FROM THE BUCCANEER OIL FIELD

The situation in the Buccaneer Oil Field is somewhat different from West Bay. The structures in the oil field support a relatively isolated reef ecosystem. If the sterols are unchanged in their composition as they pass through the food web, the steroid profiles of the various organisms would be expected to be similar to those of the plankton. This is generally the case. A major exception is the squid, which is capable of biosynthesizing sterols: the endogenous cholesterol predominates over dietary sterols in these specimens. Also, as discussed above, the barnacles contain relatively high concentrations of desmosterol. The sea urchins and polychaete worms, while capable of synthesizing sterols, also contain sterols which are of presumed dietary origin. The shrimp

are of particular interest to those who are engaged in mariculture research. These animals are incapable of the synthesis of sterols de novo, although they can accomplish the interconversion of certain sterols. This is apparently what is happening here. These specimens do not reflect the diversity of sterols found at the base of the food web (shrimp are able to convert C_{28} and C_{29} sterols to cholesterol), but the minor sterols are undoubtedly of dietary origin. In these analyses, whole shrimp were examined. However, in other analyses of *P. setiferus* from the Gulf of Mexico and mariculture facilities, we have examined individual tissues. We find that there is a great diversity of sterols in the hepatopancreas, presumably reflecting the dietary composition, and less diversity in the gonads, while the tail muscle contains mainly cholesterol and 22-dehydrocholesterol (Middleditch et al. 1980).

IMPLICATIONS FOR MARICULTURE

Our own observations have indicated that the fatty acid composition of the diet is an important consideration in obtaining ovarian maturation and spawning in penaeid shrimp (Brown et al. 1979, 1980; Middleditch et al. 1979d, 1980; Lawrence et al. 1980). Uniform results have been obtained when shrimp diets are supplemented with the bloodworm *Glycera dibranchiata*, which is rich in polyunsaturated fatty acids. These worms are expensive and are not commercially grown in Texas, so we have been seeking an indigenous alternative. Examination of the data in Table 1 reveals that several native invertebrates would be appropriate if, indeed, the fatty acid composition is as critical for maturation as our early experiments indicate. However, these data also show that caution should be exercised in selecting the particular location at which the feedstock supplements are harvested. Our results tend to confirm previous conclusions that the lipid composition of a marine invertebrate reflects that of the food web at that particular location. Indeed, we would not be surprised to find that *G. dibranchiata* obtained from some locations and at some times of the year were ineffective in promoting ovarian maturation.

While there is ample evidence that sufficient sterol is required in the diets of crustaceans, the composition of the sterols appears to be less critical than that of the fatty acids. Crustaceans have been shown to be capable of converting many different sterols to cholesterol. The only question which remains is whether it is better to present cholesterol directly to the animals in their diet or to expect them to expend additional energy in converting other sterols to cholesterol.

SUMMARY

We have obtained evidence for the homogeneity of lipids (fatty acids and sterols) in food webs where penaeid shrimp are found. These results indicate that a variety of marine invertebrates may be equally effective in promoting ovarian maturation and spawning in penaeid shrimp. These data also demonstrate that caution should be exercised in choosing a location at which the feedstock supplements are to be harvested, since the lipid composition tends to reflect that of the food web.

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